

Common molecular mechanisms of symbiosis and pathogenesis

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Symbionts and pathogens are comparable in that they colonize eukaryotic hosts. Many pathogenic and symbiotic genetic determinants are located on mobile genetic elements. Horizontal gene transfer contributes to the genetic variability of organisms, which drives the evolution of the microorganism–host interaction. Whereas pathogenic interactions result in damage or death of the respective host, symbiotic interactions are characterized by an overall benefit (Table 1). In spite of these different outcomes, similar mechanisms of pathogenesis and symbiosis exist in bacteria to facilitate successful colonization of a particular niche. The most notable mechanisms include quorum sensing and the two-component regulatory systems, which allow adaptation to the constantly changing conditions found in a new niche. Furthermore, pathogens and possibly also symbionts are able to modulate the host environment by type III secretion of effector molecules that interfere directly with host cellular functions. Of equal importance is the contribution of the host, an aspect which has been difficult to address because of the lack of experimental tractability. Clearly, features such as host immunity and susceptibility play an important role in the bacteria–host interaction and a full understanding of this interaction will therefore only be accomplished by taking an integrated approach.

Horizontal gene transfer

Genetic variability plays an important role in the evolution of pathogenic and symbiotic interactions. In addition to the chromosome, most prokaryotes possess different classes of mobile genetic elements that allow the acquisition, loss or structural change of sometimes large regions of the bacterial genome. Horizontal gene transfer represents a powerful mechanism by which the outcome of a bacteria–host interaction can be permanently altered.

Horizontal gene transfer is mediated by genomic islands, plasmids, transposons and IS elements, and phages (Table 2). Genomic islands encode functions relevant for bacteria–host interactions and are found in diverse animal and plant pathogens (where they are

Traditionally, symbiotic and pathogenic interactions were considered different manifestations of the bacteria–host interaction. However, the molecular mechanisms that mediate communication between and cellular modulation of the involved partners are quite similar. With this review we aim to contribute to a reduction of the traditional gap between symbiosis and pathogenesis research.

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known as pathogenicity islands) and also in the symbiont *Mesorhizobium loti* (a symbiosis island)¹. Plasmids can also encode many important pathogenic determinants such as toxins, type III secretion systems and invasion determinants². The plasmids of the aphid endosymbiont *Buchnera aphidicola* contain biosynthetic operons that complement the amino acid deficiency of the host³. The sym-plasmid of *Rhizobium*, which carries essential genes for symbiotic competence, might have been derived from

the plant pathogen *Agrobacterium tumefaciens*⁴. Lysogenic phages integrated in the bacterial genome are often associated with the production of virulence factors, such as toxins, in diverse pathogens and also in the symbiont *Xenorhabdus nematophilus*^{2,5}. There is also evidence for horizontal gene transfer of the IS element IS1312 from the plant symbiont *Rhizobium meliloti* to the plant pathogen *A. tumefaciens*⁶. Taken together, there is increasing evidence of horizontal gene transfer between animal and plant pathogens and between plant pathogens and symbionts. This suggests that horizontal gene transfer might be a mechanism by which genetic information can be exchanged between pathogens and symbionts in general.

Quorum sensing

Quorum sensing describes the ability of bacteria to sense cell densities and to regulate specific sets of genes accordingly⁷. It is mediated via specific signalling molecules called autoinducers (AI), which accumulate extracellularly as cell density increases. When a threshold concentration of the AI is reached, gene expression of density-dependent genes is initiated. Fundamental to this phenomenon are two genes, one synthesizing the AI (LuxI or a homologue) and the other serving as an AI receptor (LuxR or a homologue). Most but not all quorum-sensing mechanisms analysed play a specific role in symbiont– or pathogen–host interactions.

Quorum sensing regulates the virulence properties of various plant and animal pathogens. Examples include the production of cell wall degrading enzymes and carbapenem antibiotics in the plant pathogen

Table 1. Selected pathogenic and symbiotic model systems

Relevant host	Bacteria	Outcome	Proposed harm/benefit
Protozoa			
<i>Amoeba proteus</i>	X-bacteria	Pathogenic Symbiotic	Host cell killing Unknown
<i>Acanthamoeba</i> spp.	<i>Legionella</i> spp.	Pathogenic	Host cell lysis
<i>Dictyostelium</i> spp.	<i>Legionella</i> spp.	Pathogenic	Host cell lysis
Environmental amoeba	Diverse bacteria	Symbiotic	Unknown
Animals			
Sponges	Diverse bacteria	Symbiotic	Chemical defence, prevention of biofouling
Corals	<i>Symbiodinium</i> spp.	Symbiotic	Nutrients based on photosynthetic metabolism
Nematodes	<i>Xenorhabdus nematophilus</i>	Symbiotic	Killing of insect larvae
Leeches	<i>Aeromonas veronii</i>	Symbiotic	Digestion of blood
Aphids	<i>Buchera aphidicola</i>	Symbiotic	Essential amino acids
Squid	<i>Vibrio fischeri</i>	Symbiotic	Bioluminescence
Tubeworms, clams	Chemoautotrophic symbiont	Symbiotic	Nutrients based on chemoautotrophic metabolism
Animals, humans	<i>Yersinia pseudotuberculosis</i>	Pathogenic	Gastrointestinal and systemic infections, cholera
Humans	Enteric bacteria	Pathogenic	Gastrointestinal and urinary tract infections, cholera
	<i>Pseudomonas aeruginosa</i>	Pathogenic	Opportunistic infections associated with cystic fibrosis, wounds/burns, systemic infections
	<i>Bordetella pertussis</i>	Pathogenic	Whooping cough
	<i>Legionella pneumophila</i>	Pathogenic	Legionnaire's disease
Plants			
Leguminous plants	<i>Rhizobium</i> spp.	Symbiotic	N ₂ fixation
Various plants	<i>Agrobacterium tumefaciens</i>	Pathogenic	Crown gall tumors
	<i>Erwinia</i> spp.	Pathogenic	Soft rot disease
	<i>Pseudomonas syringae</i>	Pathogenic	Bacterial speck disease
Pepper, tomato	<i>Xanthomonas campestris</i>	Pathogenic	Necrotic lesions

Table 2. Mobile genetic elements in pathogens and symbionts

Genetic element	Pathogen	Encoded functions	Ref.	Symbiont	Encoded functions	Refs
Pathogenicity/symbiosis islands	<i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Shigella</i> spp., <i>Agrobacterium tumefaciens</i>	Mobility genes, type III secretion fimbriae, toxins	1	<i>Mesorhizobium loti</i>	Mobility genes	34
Plasmids	<i>Yersinia</i> spp., <i>Shigella</i> spp.	Type III secretion, invasion determinants	2	<i>Rhizobium</i> spp.	Type III secretion, N ₂ fixation, nodulation	4
	<i>Bacillus anthracis</i>	Anthrax toxin		<i>Buchnera aphidicola</i>	Amino acid biosynthesis	3
	<i>Clostridium botulinum</i>	Botulinum toxin		<i>Xenorhabdus nematophilus</i> , <i>Vibrio fischeri</i> , <i>Amoeba proteus</i>	Unknown	35–37
	<i>Staphylococcus aureus</i>	Drug resistance				
Phages	<i>Corynebacterium diphtheriae</i> <i>Vibrio cholerae</i>	Diphtheria toxin Cholera toxin	2	<i>X. nematophilus</i>	Bacteriocin	5
Transposons/IS elements	<i>Staphylococcus epidermidis</i>	Control of biofilm formation	38	<i>Rhizobium</i> spp.	Unknown	4
	<i>Neisseria meningitidis</i>	Control of capsule expression	39			

*Erwinia carotovora*⁸, the conjugal transfer of the Ti-plasmid in *A. tumefaciens*⁹, the induction of extracellular proteases in *Pseudomonas aeruginosa*¹⁰, and phenotypic changes such as motility and clumping in *Yersinia pseudotuberculosis*¹¹. Recently, a novel type of quorum-sensing mechanism has been discovered that activates genes during early- and mid-log phase¹². Examples in symbiotic model systems are the expression of rhizosphere-associated genes in *Rhizobium leguminosarum*¹³, the expression of bioluminescence genes in *Vibrio fischeri*¹⁴ and the induction of properties associated with insect pathogenesis in the symbiont *X. nematophilus*¹⁵. This strategy appears useful in light of the particular ecology of the infection process of symbionts and pathogens. The extent of physical space in an animal or plant host is limiting and therefore the excreted AI can accumulate to sufficient quantities. In addition, a critical mass of bacteria, common to both pathogenic and benign infections, and often specific host signals are required to accomplish this task.

Two-component regulatory elements

In pathogenic as well as symbiotic interactions, much of the success depends on the timeliness of the response. Because genes expressed during the infection cycle have little known relevance outside the host context, there is an 'economic' requirement to coordinate their expression. Two-component regulation is a widespread mechanism by which bacteria sense their environment and respond accordingly¹⁶. This system consists of a sensor kinase and a response regulator that mediate changes in gene expression. The environmental signals that bacteria sense upon contact with a host are similar and independent of the final outcome of the interaction. Signals that are known to play a role in virulence include pH, temperature, osmolarity and ion concentration.

Two-component systems are often linked to virulence^{16,17}. One of the best understood examples is the global virulence regulator, *phoP/Q*, of *Salmonella typhimurium*. PhoP/Q regulates at least 40 different genes including a type III secretion system located on a *Salmonella* pathogenicity island (SPI-2). The virulence of *Bordetella pertussis* is attributed to the *bugA/S* two-component system, which functions by activation or repression of specific sets of virulence genes. In *A. tumefaciens*, the *virA/G* system, which is located on the Ti-plasmid, upon induction leads to T-DNA processing and concomitant transfer to the plant genome. In the symbiosis of *R. meliloti*, the *fixL/J* two-component system induces transcription of >15 genes involved in nitrogen fixation. The symbiont *X. nematophilus* possesses a *ompR/envZ* system that permits the sensing of osmolarity¹⁸. In the unculturable symbionts of the deep-sea, hydrothermal vent tube worm *Riftia pachyptila* a functional two-component system has been cloned, however the signals remain to be elucidated¹⁹.

Type III secretion

Type III protein secretion systems deliver into host cells bacterial effector proteins that modulate host

cellular functions. They could have a general role in bacteria-host interactions as they are found in a variety of animal and plant pathogens²⁰. A functional type III secretion system has also been identified in the symbiont *M. loti*, but its role in the symbiotic interaction is unknown⁴. In most cases examined, type III secretion correlates with virulence. The genes encoding type III secretion systems often reside on genomic islands. Although the secretion mechanism appears to be relatively conserved, the biochemical activity of the effector molecule is unique for each species. The *Yersinia* protein YopH and the *Salmonella* protein SptP possess tyrosine phosphatase activity²¹ and *P. aeruginosa* ExoS exhibits ADP-ribosylation activity²². The resulting effects upon host cells are also diverse and include inhibition of phagocytosis, tissue damage, and entry into normally non-phagocytic cells. In addition, a number of phytopathogenic bacteria (*Erwinia* spp., *Pseudomonas syringae* and *Xanthomonas campestris*) use type III secretion for virulence²⁰. The fact that type III secretion systems are often located on pathogenicity islands or plasmids suggests that they have been acquired by horizontal gene transfer. The type IV secretion system is another system involved in virulence properties of various pathogens and it will be interesting to investigate whether this Sec-dependent type of secretion is also found in symbionts.

Virulence and symbiosis factors

From the analysis of the genetic and regulatory mechanisms of pathogenic and symbiotic interactions, no obvious distinguishing features are apparent. Therefore, the question arises which factors account for the many different manifestations of bacteria-host interactions. It is generally accepted that a pathogen is defined by its ability to cause disease and 'virulence factors' are the gene products responsible for infection. Invasion, and the expression of toxins, factors for colonization and gene products for the manipulation of host cell functions, ultimately lead to host damage or death. By analogy, we propose to coin the term 'symbiosis factor' to define features of a bacterium that contribute to a beneficial outcome. In addition to factors providing metabolites²³ and enzymes²⁴, this definition also includes colonization, invasion and host modulation factors²⁵. According to our view of symbiosis factors, toxins can be included if there is an overall net benefit.

Our interpretation is based on accumulating evidence that a symbiotic host initially suffers damage upon exposure to a symbiont. For example, the diploid root cells of leguminous plants undergo necrosis upon exposure to *Rhizobium* symbionts, a feature often seen in infections. The colonization of the light organ of the squid *Euprymna scolopes* with symbiotic *V. fischeri* results in induced cell death (apoptosis) of the superficial epithelial cell field²⁶. This colonization is accompanied by a transient oxidative burst in the host cells. Moreover, a host peroxidase that is closely related to a mammalian myeloperoxidase of neutrophils is induced several

hundred-fold. Furthermore, an ADP-ribosyltransferase and a ToxR homologue have recently been reported in *V. fischeri*, but their putative role in the symbiosis remains to be elucidated²⁷. Therefore, a symbiosis is more accurately viewed as reciprocal exploitation that provides net benefit for symbiont and host.

Host susceptibility

The importance of host susceptibility for the outcome of an infection is receiving increasing attention and the host responses in symbiotic interactions might be similar in many respects. In both cases, there is a need to control bacterial growth and limit damage. To illustrate the complex interplay of the bacteria–host interaction, we have developed a ‘vector model’ to help explain the different outcomes of either pathogenic or symbiotic associations (Fig. 1). It is proposed that the sum of the individual investments of the microbial partner and the respective host determines the outcome rather than the individual investments *per se*.

Free-living amoebae are valuable model systems as they allow us to address these processes in laboratory experiments. The infection of *Amoeba proteus* by *X*-bacteria is initially pathogenic and results in killing of the host. However, the adverse effects of infection gradually diminish and infected amoebae regain near-normal growth rates and become dependent on their endosymbionts²⁸. This transition shows that attenuation of the bacteria combined with a change in host susceptibility can result in symbiotic integration. In *Acanthamoeba*, both permanent mutual symbionts and highly infectious bacteria have been found^{29–31}. Analysis of the intracellular replication of *Legionella pneumophila* in *Acanthamoeba* has revealed remarkable adaptations by which a pathogen can circumvent host defences. In *L. pneumophila*, these mechanisms include the inhibition of phagolysosome fusion of the host, the reduction of the acidification of the host vacuole and cytotoxic effects²⁵. In addition, new insights are expected from systems where both bacterial and host factors can be manipulated. The amoeba *Dictyostelium discoideum* could serve as a suitable model system as it is genetically tractable. This system has recently been used for the investigation of *Legionella* pathogenicity³².

In higher organisms, pathogens and symbionts often demonstrate specific tissue tropism. Examples are the light organs of squids, the root nodules of leguminous plants or the crown gall tumour of plants. Moreover, there is increasing evidence that host defence mechanisms such as the oxidative burst are more widespread than was previously thought. For the purpose of studying the symbiotic interaction of bacteria with their hosts, it is necessary to find model systems where the bacterial partner can be separated from the host so infection assays can be performed. In addition to the model systems described in this review, the symbiosis of the medicinal leech *Hirudo medicinalis* with *Aeromonas veronii* appears particularly promising³³. Only by examining both sides of the association will a full understanding of the bacteria–host interaction be achieved.

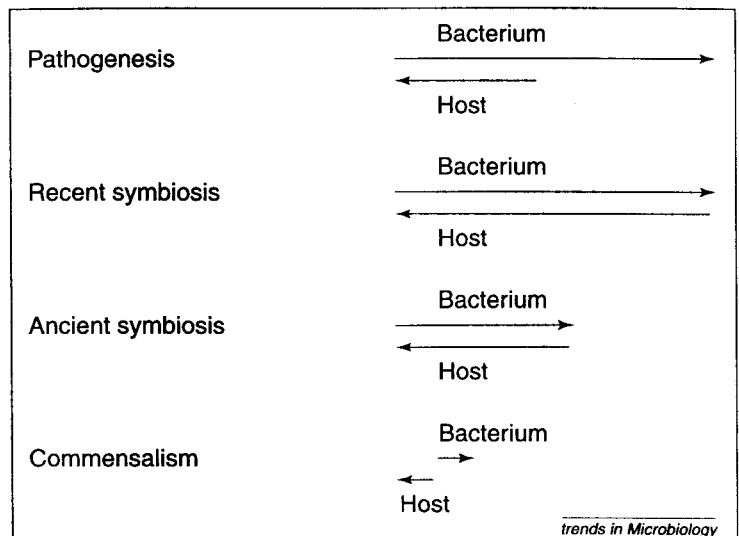


Fig. 1. Vector model of bacteria–host interactions. The model illustrates how similar mechanisms in symbiotic and pathogenic interactions can result in different outcomes. The microbial partner and the host are each represented by a vector, which is composed of the individual investments made in the association. Investments from the bacterial partner include toxins, colonization factors and effector molecules, as well as beneficial metabolites and enzymes. Investments from the host partner are receptors and host defences, as well as mechanisms for symbiont uptake and maintenance. The model states that the different outcomes of bacteria–host interactions result from the sum of the individual investments rather than from the individual investments *per se*. In pathogenic interactions, the virulence properties of the bacterium exceed the host defences. However, the change from a healthy host to a weak host (shortening of host vector) might convert a harmless bacterium into an opportunistic pathogen. Likewise, the vaccination of a host (lengthening of host vector) might neutralize the pathogenic effects of the microorganism. The loss/acquisition of a pathogenicity island (shortening/lengthening of bacterial vector) will render a bacterium avirulent/virulent. In symbiotic interactions, the opposing forces are in balance (opposing vectors of equal length). In evolutionarily recent symbioses, both host and microbial partner are characterized by costly investments to maintain balance. Here, the partners have to find each other anew with each generation. By contrast, in very ancient symbioses, these investments have been gradually reduced (shorter opposing vectors of equal length). Finally, commensalism is represented by short vectors to exemplify the minimal investments of the two involved species.

Conclusion

A review of the genetic and regulatory elements pertaining to pathogenic and symbiotic associations reveals no apparent general principles justifying a distinction between the two. Rather, the underlying strategies of bacteria–host interactions are remarkably similar in pathogens and symbionts, albeit with modified properties and functions to suit individual needs. Only when the complete interaction between a

Questions for future research

- Do pathogens and symbionts exchange genes via horizontal gene transfer?
- Do pathogens and symbionts communicate via quorum sensing?
- Do symbionts use the same secretion systems as pathogens (type III or type IV) and what might their function be?
- How do toxins contribute to symbiosis?
- Do pathogens provide useful metabolites or enzymes to benefit their host?

given pathogen/symbiont and the respective host is considered can conclusions regarding the final outcome be drawn. The proposed vector model system takes into account the individual contributions of each partner to the interaction and states that the combination of the vectors determines the final outcome. In order to study bacteria–host interactions it is crucial to find model systems where pathogens/symbionts and their hosts can be separated and where infection assays can be performed. Also, systems in which the hosts can be genetically manipulated are expected to lead to new insights. As more knowledge becomes available, the gap between pathogenesis and symbiosis research will probably be decreased.

Acknowledgements

We gratefully acknowledge Deborah S. Millikan for her insightful comments. This work was supported in part by funding from the Deutsche Forschungsgemeinschaft (Grant Ha 1434/12-1), the bmb+f Schwerpunkt 'Marine Naturstoffforschung' (03F0235A) and the Fonds der Chemischen Industrie.

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